

IN THE CLAIMS

This listing of claims replaces all prior versions, and listings, in this application.

1. (currently amended) A modified bacterial surface layer (S-layer) protein, the modification comprising the internal insertion of a heterologous polypeptide, wherein said modified protein is able to crystallize to form a crystalline monolayer and the unmodified protein is from a Gram-positive bacterium.

2. (currently amended) A protein according to claim 1, wherein[[:]]
[[(a)]] ~~the unmodified protein is from a Gram-positive or non-aquatic bacteria; and/or~~
[[(b)]] the heterologous polypeptide is a functional polypeptide or a polypeptide of interest.

3. (previously presented) A protein according to claim 1, wherein:

- (c) the protein retains most of the full length sequence of the unmodified S-layer protein;
- (d) the polypeptide is inserted at an internal location at least five amino acids from the C- or N-terminus; and/or
- (e) the modified protein has a size of from 40 to 300 kDa.

4. (previously presented) A protein according to claim 1 which:

- (f) has a crystallization or N-terminal domain that is predominantly basic, or hydrophobic;
- (g) an C-terminal domain which is predominantly hydrophilic; or
- (h) has alternating hydrophobic and hydrophilic regions.

5. (previously presented) A protein according to claim 1, wherein the heterologous polypeptide is inserted at a location in the protein so that it is:

- (i) exposed, or present on the cell surface;
- (j) present in the surface layer, or the cell wall; or

(k) is protected from external proteolytic processing or is not recognised or bound by external antibodies.

6. (previously presented) A protein according to claim 1, wherein the modified or unmodified protein:

- (l) crystallizes into an oblique lattice;
- (m) has a cell wall anchor domain;
- (n) has a pI of at least 7; and/or
- (o) is predominantly basic.

7. (previously presented) A protein according to claim 1, wherein the polypeptide comprises an antigen causing or specific for a disease.

8. (previously presented) A protein according to claim 1 which in unmodified form is from *Lactobacillus acidophilus*, *L. crispatus*, *L. helveticus*, *L. amylovorus*, or *L. gallinarum*.

9. (withdrawn) A fragment of a protein according to claim 1 which is:

- a) an N-terminal fragment or a fragment that is capable of forming a dimer with another such fragment or a trimer with two other such fragments;
- b) capable of forming dimers with another such fragment and either
 - (i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or
 - (ii) excludes an entire immunodominant or exposed loop region and is from 20 to 155 amino acids long.

10. (withdrawn) A polynucleotide encoding a protein according to claim 1.

11. (withdrawn) A vector comprising a polynucleotide according to claim 10.

12. (withdrawn) A host cell comprising, or which has been transformed with a vector according to claim 11.

13. (withdrawn) A bacteria expressing a bacterial surface layer (S-layer) protein according to claim 1 or a fragment thereof which is:

- a) an N-terminal fragment or a fragment that is capable of forming a dimer with another such fragment or a trimer with two other such fragments;
- b) capable of forming dimers with another such fragment and either
 - (i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or
 - (ii) excludes an entire immunodominant loop region and is from 20 to 155 amino acids long.

14. (withdrawn) A bacteria according to claim 13 which is a lactic acid bacteria.

15. (withdrawn) A modified bacteria other than *L. casei* or *Bacillus* which has been modified to express a heterologous surface layer (S-layer) protein, wherein said heterologous surface layer (S-layer) protein is a modified bacterial surface layer (S-layer) protein according to claim 1.

16. (withdrawn) A bacteria according to claim 15 which would not normally, or as a wild-type or in unmodified form does not, possess a surface layer.

17. (withdrawn) A modified bacteria according to claim 15 which is a *Lactobacillus* cell and/or the S-layer has its own, original, cell wall anchor.

18. (withdrawn) A bacteria according to claim 15 which is a *Lactobacillus* bacterial cell and/or the S-layer protein is from *Lactobacillus* bacteria.

Claims 19-20 (canceled)

21. (withdrawn) A modified bacteria expressing only, or homogeneously, a modified surface layer (S-layer) protein according to claim 1.
22. (withdrawn) A bacteria according to claim 21 having a genome which includes a polynucleotide encoding a modified S-layer protein, and/or where the polynucleotide encoding the normal or wild-type S-layer protein has been silenced, replaced, switched off or otherwise rendered non-expressed.
23. (withdrawn) A bacteria according to claim 22, wherein the modified S-layer protein is the sole or only S-layer protein expressed by the bacterial cell and/or the cell does not express any wild-type S-layer protein.
24. (withdrawn) A bacteria according to claim 22, wherein the S-layer protein is located on the surface of the cell wall and/or a multiplicity of S-layer proteins form an S-layer.
25. (withdrawn) A vaccine comprising a bacteria according to claim 13, a modified bacteria which has been modified to express a heterologous surface layer (S-layer) protein, wherein said heterologous surface layer (S-layer) protein is a modified bacterial surface layer (S-layer) protein or a modified bacteria expressing only, or homogeneously, a modified surface layer (S-layer) protein, wherein said bacteria has GRAS (generally regarded as safe) status.
26. (withdrawn) A vaccine according to claim 25 which is an oral or nasal vaccine and/or additionally comprises an adjuvant.
27. (withdrawn) A sheet or monolayer or 2-dimensional array comprising a plurality of bacterial surface layer proteins, at least one of which is modified protein according to claim 1.

28. (withdrawn) A solid surface, liquid-air interface, lipid film, liposome or solution comprising a sheet, monolayer or array according to claim 27.

29. (withdrawn) A solid surface according to claim 28 to which is bound one or more (macro) molecules, such as an enzyme, antibody or other binding molecule, receptor, antigen or ligand.

30. (withdrawn) A solid surface comprising a layer of S-proteins, at least a plurality of which are modified proteins according to claim 1, sandwiched between the surface and a layer of functional molecules.

31. (withdrawn) A sensor, molecular sieve or ion trap comprising a sheet, layer or array according to claim 27 or a surface comprising said sheet, monolayer or array.

32. (withdrawn) A sensor, molecular sieve or ion trap comprising a solid surface comprising a layer of S-proteins, at least a plurality of which are modified proteins according to claim 1, sandwiched between the surface and a layer of functional molecules.

33. (previously presented) A protein according to claim 1, wherein said modified protein is able to form a sheet, crystalline monolayer or two-dimensional array.

34. (previously presented) A protein according to claim 8, wherein the unmodified protein is from *Lactobacillus acidophilus* and wherein the heterologous polypeptide is inserted at a location from amino acid 1 to amino acid 290 of SEQ ID NO:2.

35. (withdrawn) A protein according to claim 34, wherein the heterologous polypeptide is inserted at a location:

- (i) from amino acid 1 to amino acid 20 of SEQ ID NO:2;
- (ii) from amino acid 35 to amino acid 55 of SEQ ID NO:2;

- (iii) from amino acid 100 to amino acid 130 of SEQ ID NO:2; and/or
- (iv) from amino acid 110 to amino acid 140 of SEQ ID NO:2.

36. (withdrawn) A protein according to claim 35, wherein the heterologous polypeptide is inserted at a location:

- (i) from amino acid 5 to amino acid 10 of SEQ ID NO:2;
- (ii) from amino acid 40 to amino acid 50 of SEQ ID NO:2;
- (iii) from amino acid 110 to amino acid 120 of SEQ ID NO:2; and/or
- (iv) from amino acid 120 to amino acid 130 of SEQ ID NO:2.

37. (withdrawn) A protein according to claim 36, wherein the position where the heterologous polypeptide is inserted at amino acid 7, 45, 114 and/or 125 of SEQ ID NO:2.

38. (withdrawn) A method of using a protein according to claim 1, said method comprising administering the modified protein or a bacteria expressing the modified protein to a human or animal by mucosal, nasal, oral, or vaginal delivery.

39. (withdrawn) A method of making a protein according to claim 1, said method comprising cultivating a host cell under conditions to provide for expression of the protein and recovering the expressed protein.